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ULTRA MICRO PROCEDURE FOR UDMH IN BLOOD

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FOREWORD

This study was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," from September to December 1961. The work was initiated by the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory, of the 6570th Aerospace Medical Research Laboratories, and represents a modification of the macro procedure described in ASD TR 61-708, "A Colorimetric Determination for 1,1-Dimethylhydrazine in Air, Blood, and Water." The assistance of Dr. Kenneth C. Back and Dr. Alan B. Cooper, Toxic Hazards Section, is gratefully acknowledged.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

An ultra micro procedure for the determination of 1, 1-dimethylhydrazine (UDMH) in blood is described. The method uses 25 microliters of serum or plasma which can be collected as capillary blood in micro hematocrit tubes and does not require deproteinization. The color reaction between UDMH and trisodium pentacyanoamino ferroate (TPF) occurs after direct treatment of the serum sample. The selection of normal commercially available control serum as a suitable blank is also discussed.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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INTRODUCTION

The colorimetric procedure for detection of 1,1-dimethylhydrazine (UDMH) in blood using trisodium pentacyanoamino ferroate (TPF) (ref. 1) routinely requires a minimum of 1 ml of whole blood. Experiments in toxicology and pharmacology using small laboratory animals often require numerous serial collections of blood from the same animal with the usual attendant technical difficulties imposed by time and limited blood volumes. Consequently, a need existed for a method which would provide accurate results using very small amounts of blood. The basic principle of the colored product formed from the reaction of UDMH and TPF was sound and was considered worthy of further use in the development of an ultra micro technique.

We considered several different approaches to the problem. Since the dilution factors inherent in any system of deproteinization, such as described in the macro procedure, impose limitations of sensitivity, a modification involving simple reduction of blood and reagent volumes would merely decrease the overall sensitivity of the test. For practical purposes then, the most desirable approach was one which would permit analysis of micro serum samples without the necessity for making a protein-free filtrate. The only significant deterrent to the direct treatment of serum or plasma with TPF reagent was the contribution of chromogenic material in individual sera to the total optical density of the test solution, a circumstance which created the problem of selecting a suitable blank. Since it was impossible to use a sample with an unknown amount of UDMH as its own blank, and since any serum contributes an appreciable increase of optical density over the color reagent itself, we investigated the magnitude of this variable in normal sera.

This report describes a simple ultra micro procedure for the analysis of UDMH in blood which can be performed on small volumes and which does not require a protein-free filtrate.

EXPERIMENTAL PROCEDURE

Human blood samples were collected from fingertip punctures, rat and mouse blood from tails, dog and monkey blood from femoral vein punctures, and rabbit blood from ear veins.

Heparinized capillary tubes were used for collection and were heat sealed. The cells and plasma were separated in a standard micro hematocrit centrifuge. Hemolysis was scrupulously avoided. No difficulty was encountered in the collection of over 150 microliters of blood from a single puncture using the usual hemolet, stylet, or needle. Approximately 75 microliters of plasma was thus available for analysis.

All colorimetric measurements were made using Coleman ultra micro equipment and accessories.

Materials

Equipment:

Ultra micro cell (1 cm light path), Auto-Vac pump, and Coleman Jr. spectrophotometer

Micro pipettes, 25 μl and 100 μl capacity

Ultra micro transfer pipettes

Microhematocrit centrifuge

Heparinized capillary tubes

Plastic micro titration cups (disposable)

Reagents:

Buffer, pH 5.4: 4.8 grams citric acid and 19.63 grams disodium acid phosphate dissolved in 1.0 liter of distilled water

Trisodium pentacyanoamino ferroate (TPF): 0.1 percent aqueous solution, prepared fresh daily

Method

For each determination set up 3 plastic micro titration cups. Identify these as UNKNOWN, NEGATIVE CONTROL BLANK, and REAGENT BLANK.

Pipette the following quantities of ingredients:

	UNKNOWN	NEGATIVE CONTROL	REAGENT BLANK
Plasma or serum	25 μl	25 μl*	
Buffer	100 μl	100 μl	125 μl
TPF reagent	100 μl	100 μl	100 μl

* Commercially available normal serum control is satisfactory.

Allow color development to proceed for 40-60 minutes at room temperature.

Transfer a suitable aliquot of reagent blank to the spectrophotometric ultra micro cell and set at 100 percent transmittance using 500 mμ wave length. Evacuate the cell carefully and completely.

Transfer aliquot of negative control blank to the micro cell and record the optical density reading. Evacuate the cell.

Transfer aliquot of unknown to the micro cell and record the optical density reading.

Calculation

O. D. unknown - O. D. negative control blank = corrected O. D. of unknown.

Convert the corrected O. D. of the uknown to μg of UDMH per ml of plasma by referral to a standard calibration curve.

If preferred, a convenient single concentration of UDMH in plasma or serum may be prepared and used as a standard in the calculation of μg/ml by application of the formula:

$$\frac{\text{O. D. unknown - O. D. negative control blank}}{\text{O. D. standard - O. D. negative control blank}} \times \text{Concentration of Standard (in } \mu\text{g/ml)} = \text{Concentration of Unknown (in } \mu\text{g/ml)}$$

DISCUSSION

Hemolysis, excessive amounts of bile pigments, lipid materials, or other chromogenic substances will increase the total apparent optical density of serum, and will prevent an accurate determination of UDMH content by the method as described. Such conditions occur infrequently, however, and would not be expected to present a problem of any magnitude. If such interference is exhibited, the macro procedure with deproteinization must be used.

Ten replicate serum samples analyzed both by the macro method and ultra micro modification proved that recovery, reproducibility, and sensitivity were not sacrificed in the simplified direct treatment procedure.

Analyses of 50 different blood plasma samples from nonexposed subjects revealed a relatively constant optical density reading of 0.194 with a standard deviation of 0.023, a value which is well within the instrumental limits of error of most spectrophotometers. We are confident that using this constant on a routine basis provides a valid UDMH negative serum correction factor with which to perform the ultra micro procedure, either as a mass screening technique for humans or as a method for analysis of limited volume blood samples from small experimental animals. This procedure also obviates the practice of "pooled sample" analysis which all too often serves to mask small but significant individual variations.

Reading of a serum-buffer blank against the reagent blank does not provide a satisfactory substitute for the negative control blank. Apparently some normal constituent of the serum reacts with the TPF reagent to produce an increase in O. D. over that of serum-buffer alone (no TPF). This reaction is relatively constant and predictable, whereas individual serum color intensity is exceedingly variable. Therefore, a practical optical density correction factor may be established for negative control serum and routinely used in the calculation of UDMH content in unknown samples by the ultra micro method.

REFERENCE

1. Pinkerton, Mildred K., J.M. Lauer, P. Diamond, and A.A. Tamas, A Colorimetric Determination for 1,1-Dimethylhydrazine in Air, Blood, and Water, ASD Technical Report 61-708, Aeronautical Systems Division, Wright-Patterson Air Force Base, Ohio, December 1961.

Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. AMRL-TDR-62-120, ULTRA MICRO PROCEDURE FOR UDMH IN BLOOD. Final report, Oct 62, iii + 4 pp. incl. 1 ref.
Unclassified report

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1. UDMH
2. Methyl hydrazines
3. Ferrates
4. Blood plasma
5. Colorimetric analysis

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III. Pinkerton, Mildred K., Thomas, A.A.
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